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Effect of Rizolex on *Rhizoctonia solani* Kuhn isolates and biocontrol fungus *Trichoderma harzianum* Rifai *in vitro*

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Abstract

A study was carry out to detect effect of Rizolex(Tolclofosmethyl) on growth of some isolates of a pathogenic fungus *Rhizoctonia solani* Kuhn and growth ,sporulation and spore germination of biocontrol agent *Trichoderma harzianum* Rifai *in vitro* .Results showed that Rizolex was strongly growth inhibition of *R. solani* (100%) at all concentrations (0.025 , 0.05,0.25 and 0.5 ppm) ,while it's less inhibited to *T. harzianum* (65.60% - 79.66%) compared with *R. solani* .Result of sporulation of *T. harzianum* showed that Rizolex reduce number of formed spores about 40.8% - 49.6 % compared with control . Germ tubes length of spores of *T. harzianum* decreased 30.6% , 34.88% and 43% by effect of fungicide concentrations 0.05, 0.25 and 0.5 ppm respectively .Also the percentage of spores germination were reduced 42% , 40.5% and 36.6% at same concentrations .While ,there are significantly different in spores germination of *T. harzianum* between concentrations 0.05 and 0.5 ppm (42% and 36.6%) .

Introduction

Rhizoctonia solani Kuhn probably causes different type of diseases to a wider plant cultivars over a large part of the world and under more diverse environmental condition compared with other plant pathogens (1) .Damping off disease is one of the most important diseases affected in the yield potential of many crops that caused by *R.solani* (2). Fungicides ,such as pentachloronitrobenzene (PCNB) ,are commonly used that are highly specific against *R.solani* (3) .The repeated use of these pesticides has not only polluted the environment, but many pesticides have also become useless owing to the development of resistance among the target organisms. One possible alternative to

synthetic fungicide is the use of biological control agents (4,5). However, biocontrol is not often used in commercial agricultural production because control of plant diseases with microbial agents has been less effective and reliable than with synthetic fungicides (6) .The fungus *T. harzianum* has been used a biocontrol and shown to attack rang of economically important soil-born fungal plant pathogens (7) . In soil ,*T.harzianum* strains have been used as antagonists against several plant pathogenic fungi , for example , *Rhizoctonia solani* (8) , *Botrytis cinerea* and *Sclerotium rolfsii* (9) and *Fusarium* (10) .*T.harzianum* used together with fungicide Benomyl to control of *R. solani* (11) , and with fungicide PCNP to control a same pathogen (12) , also using *T. verens* with Metalaxyl (13).Therefore this study aimed to detect effect of Rizolex on growth of *R.solani* isolates and growth ,sporulation and germination of spores of *T.harzanium* as indicator to microbial balance between pathogen and biocontrol agent after using Rizolex.

Materials and Methods

Isolation of fungi

Four isolates of *R.solani* Kuhn were isolated from infected plant : cotton seedling , eggplant seedling , tuber surface of potato and stem of feverfew.The isolates were isolated by sterile infected parts of plant by chlorex 1% and cultured at plates contain 20 ml potato dextrose agar (PDA) .The fungus *T.harzianum* was isolated from biofungicide Tahiidi (14) .

Preparation of Rizolex concentrations

Concentrations of fungicide of Rizolex 50%wp(Tolclofosmethyl) (manufactured by Sumitomo Chemical Co. Japan) including 0.025, 0.05, 0.25 and 0.5 ppm were prepared by adding 6.25, 12.5, 62.5 and 125mg active ingredient (a.i.) to 100 ml sterile distilled water for prepare stock suspensions that using in experiments. Concentrations were based on ppm of active ingredient of fungicide (15) .

Effect of Rizolex concentrations on growth of four *R.solani* isolates and *T. harzianum* in plate

A concentrations of Rizolex were obtained by adding appropriate amount of stock suspensions (1 ml) to 249 ml portions of autoclaved PDA cooling to about 50C◦. Approximately 20ml of un amended (control) or amended PDA were poured into each petri dishes (9cm diameter) , three plates as replicates for each treatment . After solidification of the medium each plate was inoculated centrally with a

mycelial disc (5mm diameter) taken from the outer margins of actively growing mycelium from PDA cultures of each isolate by sterile cork borer. Plates were incubated at 25 ± 2C◦ and colony diameters were measured after 5days .When the untreated control had just covered the plate (15).Percentage of growth inhibition (I%) was calculated according to the following formula suggested by Topps and Wain (1957) : I%=[(A-B)/A]×100 where A= mean diameter of growth in the control and B=mean diameter of growth in the given treatment (16) .

Effect of Rizolex concentrations on sporulation of *T.harzianum*

The concentrations of Rizolex 0, 0.05, 0.25 and 0.5 ppm were prepared and added to autoclaved PDA cooling to about 50C◦ as previous experiment .PDA with fungicide concentrations and PDA without fungicide of each treatments were poured into each of three petri dishes as replicates . After solidification of the medium ,the plates were inoculated centrally with a mycelial disc(5mm diameter) taken from the outer margins of recent culture of *T.harzianum* (3days old).The plates were incubated at 25 ± 2C◦.When mycelium was fill of petri dishs, 2disc (5mm diameter)for each treatment from far 1cm of center of petri dish .Each disc was added to volumetric flask (50ml) have 10ml sterile distilled water . Number of spores were counted by heamocytometer (17).

Effect of Rizolex on spores germination and germ tubes of *T. harzianium*

Appropriate amounts of stock suspensions of Rizolex were added to 249 ml of water agar medium to obtain concentrations 0, 0.05, 0.25 and 0.5 ppm. Approximately 20 ml of medium was poured into each petri dish ,three plates for each treatment as replicates. 1disc (5mm diameter) of colony of *T.harzianum* was added to 10 ml sterile distilled water. Number of spores of *T.harzianum* were counted by haemocytometer and it's amended to 10^4 /ml . Spores suspension 0.5 ml was added to each plate for each treatment. The plates were incubated at 25 ± 2C◦ .After 24 hours the germination stopped by drops of formalin, also number of germinated spores and non germinated spores were counted and converted as percentage in two disc (5mm diameter) of each petri dish (18) .Length of germ tubes were measured by ocularmicrometer in two disc.

Statistical Analysis

All experiments were designed complete randomized design and data analyzed by using least squares analysis of variance (ANOVA), Least

Significant Difference (L. S. D.) test was used at the 1% level of significance (19).

Results

The results of this study showed that Rizolex was highly effective on radial growth of tested fungi (Table 1).There are difference between *Rizoctonia solani* and *T.harzianum* in response to efficacy of Rizolex , then isolates of *R.solani* were inhibited completely at all concentrations , while *T.harzianum* could growing and reached with different percentage of inhibition 65.60%, 68.66%, 70.67% and 79.66% according to concentration of Rizolex .

Table1. Percentage of inhibition (%) of Rizolex concentrations to radial growth of *R.solani* isolates and *T.harzianum*.

Concentration of Rizolex** (ppm)	Isolates of *R.solani*				*T.harzi-anum* %	Means %
	potato %	feverfew %	cotton %	eggplant %		
Control	0	0	0	0	0	0
0.025	100*	100	100	100	65.60	93.12
0.05	100	100	100	100	68.66	93.73
0.25	100	100	100	100	70.67	94.13
0.5	100	100	100	100	79.66	95.93
Means %	80	80	80	80	56.92	

L.S.D.p=0.01 for isolates =0.53
L.S.D. p=0.01 for concentrations =0.53
L.S.D. p=0.01 for concentration ×isolates =1.2
***Each number is mean of trireplicate**
**** Active ingredient (a.i.)of Rizolex**

Also the results of sporulation showed that all concentrations of Rizolex reduced number of spores to 22.83×10^5, 26.1×10^5 and 26.83×10^5 compared with control (45.33×10^5) (Fig.1).

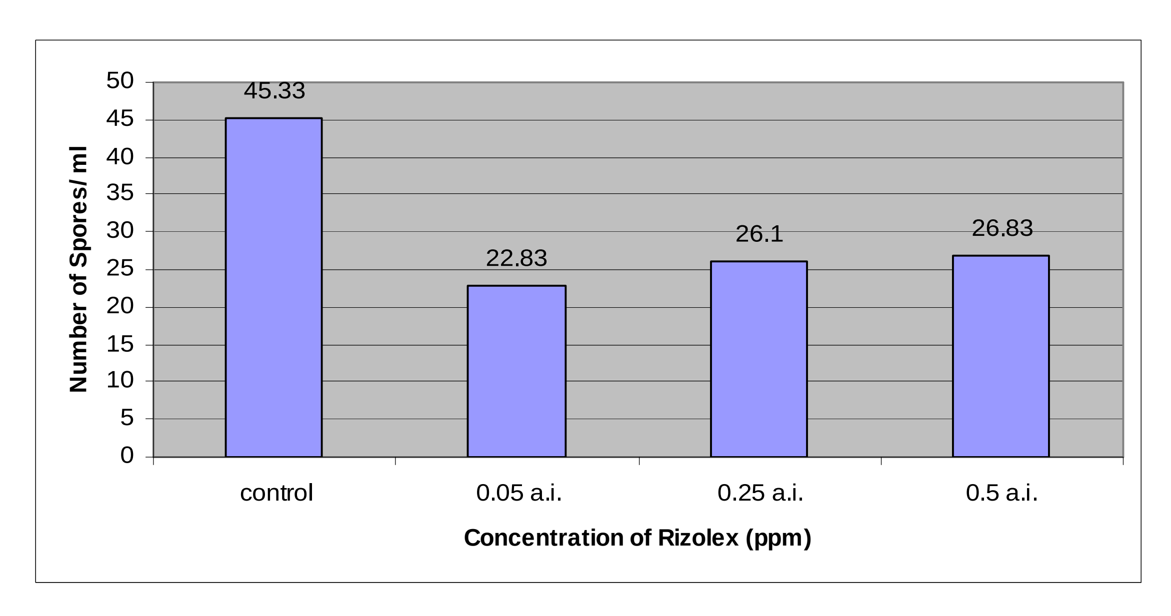


Fig.1.Effect of Rizolex concentrations on sporulation of *T.harzianum*.
***L.S.D p=0.01 =10.1**
***Each number is mean of trireplicate ×10^5**
**** Active ingredient (a.i.)of Rizolex**

The results of Rizolex effect on length of germ tubes of spores showed that concentrations 0.05, 0.25 and 0.5 ppm decreased length of germ tubes 30.6%, 34.88% and 43% respectively compared with 124.5 micrometer in control treatment (Fig.2).

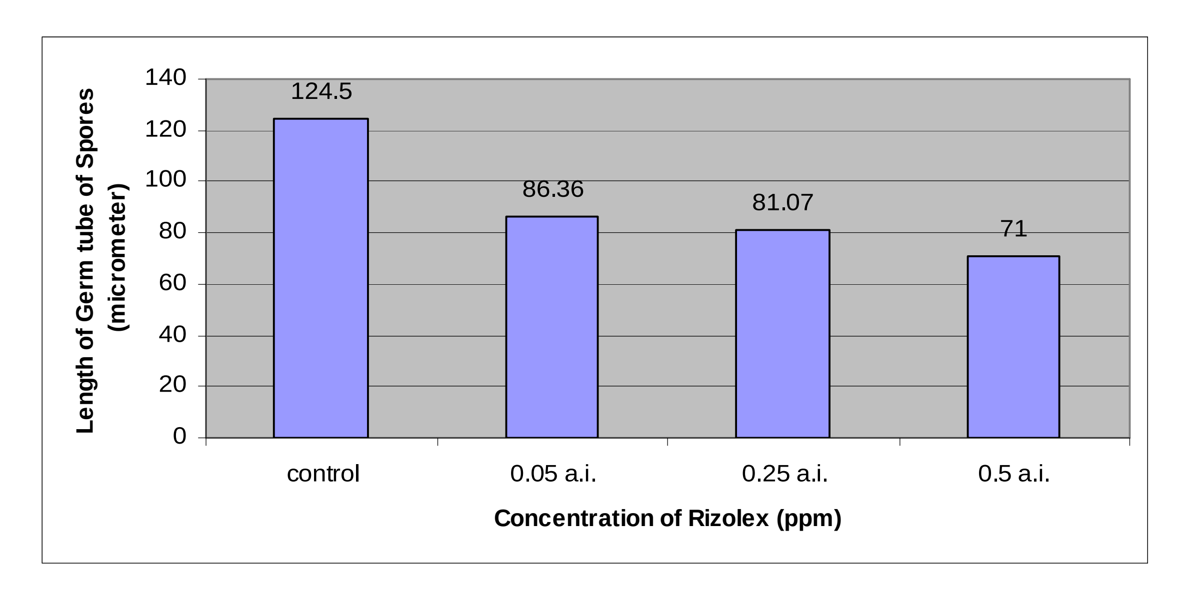


Fig.2. Effect of Rizolex concentrations on length of germ tubes of *T.harzianum* spores .

***L.S.D.p=0.01 =23.4**
***Each number is mean of trireplicate**

While the percentage of spores germination were reduced to 42%, 40.5% and 36.6% in concentrations of Rizolex 0.05, 0.25 and 0.5 respectively. Also there are significantly difference between the concentrations 0.05 and 0.5 ppm which were 42% and 36.6% respectively (Fig.3).

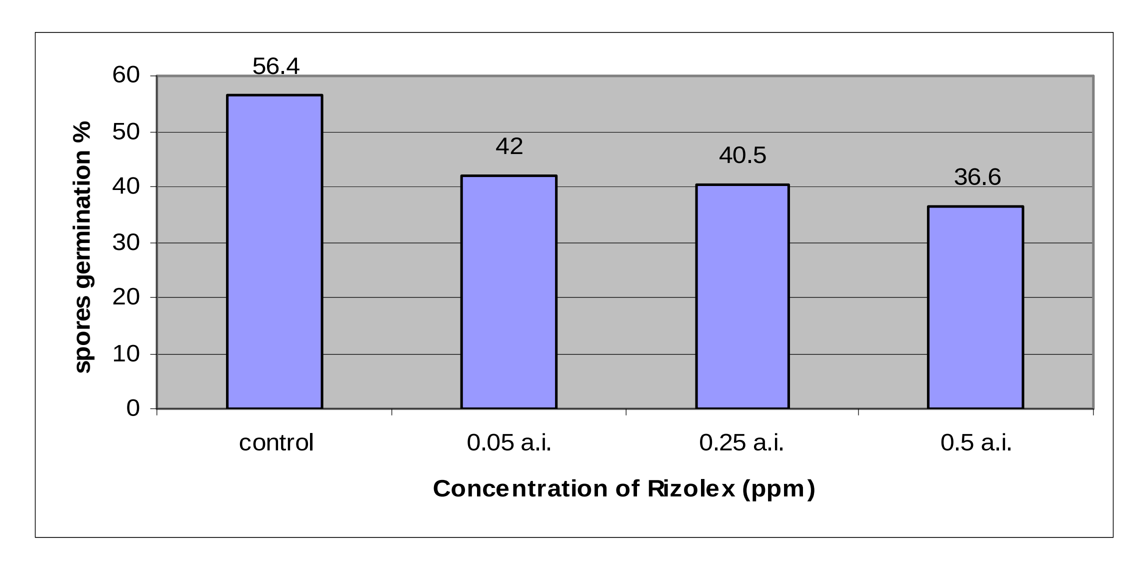


Fig.3. Effect of Rizolex concentrations on spores germination of *T.harzianum.*
***L.S.D. p=0.01=6.34**
***Each number is mean of trireplicate**

Discussion

In the present study , considerable variation in efficacy of Rizolex fungicide against *Rhizoctonia solani* isolates and *T.harzianum* was observed .Completed inhibition of radial growth of *R.solani* isolates by all concentrations was recorded , also this results indicate that didn't found isolate of *R.solani* was resistant to Rizolex .Therefore ,Rizolex is consider as very effect on *R.solani*. Machowicz-Stefaniak (1994) classified some fungicides as very fungicidal ,falling into group one of the fungicidal activity as it's ED50(Effective Dose for 50%) if it's below 1 ppm of active ingredient (a.i.) (20) .While, *T.harzianum* appeared lower susceptibility than *R.solani* isolates in spite ,efficiency of Rizolex against *T.harzianum* .I think that the variation between *R.solani* and *T.harzianum* in susceptibility to Rizolex may be using for control *R.solani* by Rizolex and biocontrol fungus *T.harzianum* as integrated pest management (IPM) . Previous studies reported using benlate with *T. harzianum* to control *R.solani* (11) , also pentachloronitrobenzene (PCNB) with *T.harzianum* as IPM against *R.solani* (12,21).In other hand Machowicz-Stefaniak *et al.* (1998) investigating 11 fungicide containing various active substances observed that fungicides in concentration of

100 ppm inhibited growth of *T.harzianum* colonies. Susceptibility of fungus to the preparations researched differed, depending on the kind of fungicide. However for each preparation a decrease in concentration of active ingredient was related to a loss of fungistatic activity .Although most chemical compounds accumulating in soil undergo decomposition and detoxification , there are reports disturbed microbiological balance in soil (22). Apply high doses of benzimidazol fungicides against *Gaeumonnomyces graminis* fungus increases the cereal infection with *Rhizoctonia* root rot .This phenomenon is a result of deterioration of antagonistic micrflora ,mainly *Trichoderma* and *Penicillium* genera fungi (23) .Therefore ,we must study side effect of fungicide on biocontrol agent before using it to control any disease .

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